



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁶ : G01N 33/566, 33/50	A3	(11) International Publication Number: WO 99/51777 (43) International Publication Date: 14 October 1999 (14.10.99)
(21) International Application Number: PCT/US99/07566 (22) International Filing Date: 6 April 1999 (06.04.99) (30) Priority Data: 60/080,915 6 April 1998 (06.04.98) US (71) Applicant (for all designated States except US): BUNSEN RUSH LABORATORIES, INC. [US/US]; 4 Nobscot Road, Newton, MA 02159-1323 (US). (72) Inventor; and (75) Inventor/Applicant (for US only): LERNER, Michael, R. [US/US]; 3725 Colgate Avenue, Dallas, TX 75225 (US). (74) Agent: GATES, Edward, R.; Wolf, Greenfield & Sacks, P.C., 600 Atlantic Avenue, Boston, MA 02210 (US).		(81) Designated States: CA, JP, US, European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE). Published <i>With international search report.</i> (88) Date of publication of the international search report: 9 December 1999 (09.12.99)
(54) Title: DIRECTED EVOLUTION BIOSENSORS (57) Abstract <p>The invention exploits the evolutionary principles responsible for the development of the broad spectrum general odorant detector system, to create a G-protein coupled receptor (GPCR) based system capable of detecting and discriminating between thousands of chemicals. The means is to subject a defined set of receptors such as G-protein coupled receptors, tyrosine kinase receptors, and/or ion channels, to the types of evolutionary forces that have created the array of approximately 1,000 natural receptors used in general olfaction by higher animals. This goal is accomplished by "directed evolution-in-a-test-tube" by imposing very high rates of mutation and extremely strict selection criteria to create a sensor. The novel sensor is selected using a sensitive melanophore-based functional bioassay. Stimulation of the sensor upon interaction with chemical signatures derived from ordinances will result in a calcium ion flux rapidly detectable as a fluorescent signal.</p>		

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INTERNATIONAL SEARCH REPORT

International Application No.
PCT/US 99/07566

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 G01N33/566 G01N33/50

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 95 02823 A (BRANN M.R.) 26 January 1995 (1995-01-26) page 5, line 25 - page 9, line 29 page 23, line 28 - page 28, line 18; claims ---	1-3
X	WO 93 23431 A (BAYLOR COLLEGE OF MEDICINE) 25 November 1993 (1993-11-25) page 3, line 14 - page 6, line 16; claims 1-25; figure 1; examples 2,10,11,14 --- -/--	1-3, 11-13, 15, 16, 23,25

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

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"A" document defining the general state of the art which is not considered to be of particular relevance

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"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search

23 August 1999

Date of mailing of the international search report

14. 09. 99

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INTERNATIONAL SEARCH REPORT

Inter. Appl. Application No.

PCT/US 99/07566

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5 462 856 A (M.R. LERNER ET AL.) 31 October 1995 (1995-10-31) cited in the application abstract column 12, line 43 - column 15, line 28; claims ---	26,27
X	T.S. MCCLINTOCK ET AL.: "Functional analysis by imaging of melanophore pigment dispersion of chimeric receptors constructed by recombinant polymerase chain reaction" BRAIN RESEARCH (ISSN: 1385-299X), vol. 2, no. 1, 1 December 1997 (1997-12-01), pages 59-68, XP002112945 n1 abstract, "results", "discussion" ---	1-4,6-9, 26-28
Y	abstract, "results", "discussion" ---	11,17,30
X	LERNER M.R.: "Tools for investigating functional interactions between ligands and G-protein-coupled receptors" TRENDS IN NEUROSCIENCES, vol. 17, no. 4, 1994, pages 142-146, XP002113036 ISSN: 0166-2236 the whole document ---	11-21
Y	ZICCARDI M. ET AL.: "Direct detection of dioxins and related chemicals in small quantities of human serum using a recombinant cel bioassay" ORGANOHALOGEN COMPOUNDS, vol. 31, 1997, pages 215-218, XP002112946 ISSN: 1026-4892 the whole document ---	11,17,30
A	KRYSTEK S.R. ET AL.: "Mutation of peptide binding site in transmembrane region of a G protein -Coupled receptor accounts for endothelin receptor subtype selectivity" JOURN. BIOLOGICAL CHEMISTRY, vol. 269, no. 17, 1994, pages 12383-12386, XP002112947 US (ISSN: 0021-9258) the whole document ---	11-17
A	WO 97 42490 A (LION LABORATORIES) 13 November 1997 (1997-11-13) cited in the application page 3, line 18 - page 6, line 2 page 9, line 12 - page 11, line 1; claims ---	23,31,32

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INTERNATIONAL SEARCH REPORT

Inter national Application No
PCT/US 99/07566

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
E	<p>WO 99 19506 A (IXSYS, INC.) 22 April 1999 (1999-04-22) page 3, paragraph 137 - line 16; claims -----</p>	1

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 99/07566

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claims Nos.: 5,17,25,30 (partially)
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
see FURTHER INFORMATION sheet PCT/ISA/210

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Claims Nos.: 5,17,25,30 (partially)

The term "impurities" is vague and can only be given a technical meaning within a specific context which is absent from claims 5,17,25 and 30.

The applicant's attention is drawn to the fact that claims relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No.

PCT/US 99/07566

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9502823 A	26-01-1995	AT 177535 T	15-03-1999
		AU 679253 B	26-06-1997
		AU 7333094 A	13-02-1995
		CA 2167048 A	26-01-1995
		DE 69417037 D	15-04-1999
		EP 0708922 A	01-05-1996
		ES 2129658 T	16-06-1999
		IL 110298 A	11-04-1999
		JP 9500023 T	07-01-1997
		NZ 269442 A	24-06-1997
		US 5707798 A	13-01-1998
		US 5912132 A	15-06-1999
WO 9323431 A	25-11-1993	US 5364791 A	15-11-1994
		AU 685054 B	15-01-1998
		AU 4241793 A	13-12-1993
		AU 6065198 A	02-07-1998
		CA 2135644 A	25-11-1993
		EP 0745121 A	04-12-1996
		JP 7509694 T	26-10-1995
		US 5874534 A	23-02-1999
US 5462856 A	31-10-1995	CA 2087614 A	20-01-1992
		EP 0539518 A	05-05-1993
		JP 6502757 T	31-03-1994
		WO 9201810 A	06-02-1992
WO 9742490 A	13-11-1997	US 5753511 A	19-05-1998
		AU 2998497 A	26-11-1997
		CA 2253447 A	13-11-1997
		CN 1217789 A	26-05-1999
		EP 0897532 A	24-02-1999
WO 9919506 A	22-04-1999	AU 9796698 A	03-05-1999